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(54) Title: ENCAPSULATION OF BIOLOGICAL MATERIALS IN SEMI-PERMEABLE MEMBRANES

(57) Abstract

Material such as biological material is encapsulated within a semi-permeable membrane by suspending the material in a medium which comprises an effective amount of a gelling inducer; forming said suspension into a droplet of a size sufficient to envelop said material; and then forming a discrete capsule by contacting the outer surface portion of the droplet with a gelling solution comprising an effective amount of a gel forming polymer which gels upon contact with said gelling inducer.

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ENCAPSULATION OF BIOLOGICAL MATERIALS IN SEMI-PERMEABLE MEMBRANES

Technical Field

The present invention relates to encapsulated products. More particularly, the present invention relates to a process for the encapsulation of bioactive materials in semi-permeable membranes of gel forming polymers.

Background Art

Encapsulation processes are finding increasing use in a variety of areas of biotechnology. Such processes are used to encapsulate various materials such as enzymes, hormones, drugs, adsorbents and cells which can then be used in bioreactors, artificial organs, bioseparation systems, controlled drug-release systems, and so forth. Prior art processes often require harsh conditions such as the use of non-aqueous solvents, extremes of pH, or high temperature. Such techniques are inherently unsuitable for encapsulating delicate biological materials such as live cells and labile proteins. Ideally, encapsulation techniques for biological materials should use mild conditions and a membrane material which is inert and non-toxic to the material being encapsulated. Also, the encapsulation technique should provide a semi-permeable membrane and allow for adjustment of membrane thickness and membrane pore size. Preferably, the charge on the membrane should be adjustable to suit different applications. The membrane should also be strong enough to withstand liquid-shear or the friction effects arising out of agitation.

A well known membrane encapsulation method is the poly (L-lysine) - alginate membrane method which involves formation of a polyelectrolyte membrane complex. In this method the mixture of bioactive material and sodium alginate is extruded through a droplet forming device into a buffer containing calcium chloride. The Ca^{+2} cations cross-link the alginate matrix almost instantaneously to form gel beads. The beads are then treated with poly-l-lysine to displace the calcium ions in the outer layer to form a polyelectrolyte-complex membrane. Calcium alginate gel in the interior of the bead is then liquified using a calcium chelating agent. Membrane encapsulation methods do not suffer from the problems observed in gel entrapment.

In animal cell culture applications it has been observed that cells grow profusely within membrane capsules to reach tissue like densities. However this method involves a large number of processing steps. This method also provides a membrane which is charged due to its polyelectrolyte nature. Also, the membrane has relatively poor mechanical strength and poor chemical stability in the presence of electrolytes such as heparin, polysulfonic acid and polyphosphoric acids which interact more strongly with alginate or poly (L-lysine). Furthermore, liquified alginate remains within the capsule. Alginate can interfere with the functioning of biomaterial by complexing with multivalent ions or other charged macromolecules. Alginate can also adsorb on positively charged surfaces and cause fouling.

Thus, there remains a need for an improved process for encapsulation of bioactive materials and it is an object of the present invention to provide such an improved process. Further understanding of this invention will be had from the following description and claims. All parts and percentages herein are by weight unless otherwise indicated.

Disclosure Summary of the Invention

In accordance with the present invention, the desired material is encapsulated within a semi-permeable membrane by a process comprising the steps of:

(A) suspending said material to be encapsulated in a medium which is compatible with the material and which comprises a small, effective and diffusible gelling inducer such as Ca^{+2}, K^{+}, polyphosphate, etc. Optionally, the medium may also contain a viscosity enhancer;

(B) forming said suspension into a droplet of a size sufficient to envelop said material, said droplet having an outer surface layer;

(C) gelling said droplet to form a discrete capsule by contacting said outer surface layer with a gelling

solution comprising an effective amount of a gel forming polymer which gels on contact with said gelling inducer.

Optionally, the outer surface layer of the capsule can be coated with a second polymer to form a composite membrane. Alternatively, the gelling solution comprises an effective amount of a second gel forming polymer in addition to the first gel forming polymer. The capsules formed after the gelation of the first polymer can be removed from the polymer solution. The physico-chemical conditions can be altered to induce the gelling of the second polymer entrapped within the capsule membrane.

Detailed Description of Modes for Carrying Out Preferred Embodiments of the Invention

In accordance with the present invention, a gel forming polymer system is used to form a semi-permeable membrane encapsulating various materials. It will be appreciated that the process of this invention is particularly well suited for use in encapsulating biological materials. Unlike most of the known processes for encapsulating biological materials the present process ensures that most of the biological material never comes in contact with the gel forming polymer. The biological material stays within its original environment in the suspension. Thus, the description of the preferred embodiments of this invention is in the context of encapsulating biological materials. However, the process of this invention can also be used to encapsulate other materials and such other uses are contemplated to be within the broad scope of this invention.

The biological material to be encapsulated can be tissue, organelle, plant or animal cells, delta cells, whole islet of Langerhans, hepatocytes, bacteria, algae, fungi, viruses, proteins, pharmaceutical compounds and so forth. The material must be of a size small enough to be suitable for encapsulation by the droplet method of this invention but can vary widely in diameter from less than a micron to several millimeters. The present process allows viable cells to be encapsulated in a semi-permeable membrane allowing cells access to

nutrients and other substances necessary for viability but protecting cells from substances having a molecular weight above a selected size such as antibodies, toxins and bacteria. Thus the biological can be maintained in a viable state for an extended period of time.

The biological material is first suspended in an aqueous medium which is physiologically compatible with the material. The medium should comprise required nutrients, be without toxic substances and have a suitable pH as, for example, a typical buffered solution. The medium also comprises an effective amount of a gelling inducer. The gelling inducer is of a type and in an amount effective to diffuse outwardly and cause the gel forming polymer to gel when coming into contact therewith as described in more detail hereinafter. Optionally, the aqueous medium also comprises a viscosity enhancer such as dextran, hyaluronic acid, polyethylene glycol, starch etc.

The suspension of material being encapsulated is formed into droplets of a size sufficient to envelop the material by, for example, dropping the suspension through a fine nozzle, capillary tube or hypodermic needle. This method is amenable for delicate biological materials. Alternatively, the material being encapsulated can be pelletized using a punch-press type apparatus or using a pellet mill for large scale applications. The outer surface layer of the droplet or pellet is almost instantly provided with a gelled semi-permeable membrane by contacting the outer surface layer with the gel forming polymer as by, for example, dropping the droplet into a vessel containing a rapidly stirred solution of the gel forming polymer(s). The gel forming polymer is contacted by the gelling inducer to almost instantaneously form a semi-permeable membrane encapsulating the droplet.

The gel forming polymer can be any non-toxic water soluble gel forming polymer which forms a gel upon contact with a gelling inducer. Optionally, the gel forming polymer is an ionotropic gel forming polymer such as a water soluble polysaccharide. Suitable polysaccharides include those typically extracted from vegetable matter and include sodium alginate, guar gum, gum arabic, charagunan, pectin, tragacanth gum, xanthan gum, and deacylated chitin (chitosan). Upon contact with gel inducers the polysaccharide molecules form a water-insoluble shape-retaining gel capsule.

It is an advantage of this invention that the gel forming polymer comes in contact only with the outer surface portion of the droplet before gelling. There is thus little, if any, effect of the polymers on the material being encapsulated. It is another advantage that this process obviates the need for an initial gel-entrapment step for capsule formation.

If the type of membrane formed using the initial gelation is adequate for the particular bioprocessing application desired, the capsules can be recovered from the gelling solution and equilibriated with the desired media. However, the mechanical and chemical properties of the capsule membrane can be further altered to suit different bioprocessing and biomedical applications.

In order to alter the membrane a second gel forming polymer can be used to impart altered properties to the membrane such as mechanical strength, chemical stability, pore size and/or surface charge. The second polymer can be another polyelectrolyte having opposite charge to that of the first polymer. In this case the second polymer can be coated on the outer surface of the capsule to complex with the initial gel membrane. The resulting polyelectrolyte complex imparts greater chemical stability to the capsule. For example, sodium alginate capsules can be coated with polycations such as poly (L-lysine), polyethylene-imine, chitosan or acrylate/methacrylate copolymers (Eudragit® RL 100 from Rohm GmbH, Darmstadt, FRG) to form capsules having composite membranes. It is possible to obtain capsules with desired charge characteristics on either side of the membrane.

Alternatively, the gel forming solution comprises a solubilized second gel forming polymer in addition to the first polymer. The almost instantaneous gelling of the first gel forming polymer to form the initial membrane entraps the second gel forming polymer in the membrane. Also due to high viscosity some polymer solution may adhere to the exterior of the capsule surface when it is removed from the gelling solution. The capsules can be placed in an oil medium or in a buffer solution containing gelling inducer for the first and/or polymer. Both of these approaches curtail any loss of the thin liquid polymer solution film covering the capsule. It will be apparent to those skilled in the art that the physico-chemical

conditions of the capsule can be altered in various ways to induce gelling of the second polymer.

Optionally, the second gel forming polymer can be a thermal gel forming polymer. Thermal gel forming polymers undergo gelation when their temperature is lowered below their gelation temperatures and generally have chemical and mechanical properties which are superior to ionotropic gels. Though widely used in gel entrapment, currently no method exists for membrane encapsulation using thermal gels. A wide variety of thermal gel forming polymers can be used in the present invention, including, for example, agarose and kappa-carrageenan.

If desired, the first polymer component of the composite membrane can be removed by means well known in the art. For example, if the first polymer is an ionotropic gel forming polymer, its dissolution can be achieved by contacting the capsule with a chelating agent after the gelling of the second polymer is complete.

It will be appreciated that the present process is versatile and subject to substantial variation. The membrane can be formed using a wide variety of available gel forming polymers. Membranes of different characteristics can be obtained by manipulating the type and the concentration of the polymers. Aqueous thermal gels such as agarose, κ-carrageenan, or gelatin may be employed to encapsulate delicate materials such as live cells and labile proteins. If the material being encapsulated is relatively stable in the presence of organic solvents, reactive cross-linking agents and extremes of pH for short durations of time, the list of polymers useful herein can be further expanded to include precipitation gels (eg. cellulose acetate), polycondensation gels (eg. epoxy and polyurethanes) and copolymerized gels (eg. polyacrylamides). The capsules formed using these polymers can be used to encapsulate adsorbents, drugs and stable enzymes in membranes of greater structural rigidity and chemical inertness.

EXAMPLE 1

This example illustrates formation of calcium alginate capsules containing material to be encapsulated. A solution containing 0.8% sodium alginate (Sigma A 7128, type IV) is prepared and kept stirred using a magnetic stirrer at room temperature. An aqueous suspension containing the material to be encapsulated is prepared in 0.1M HEPES buffer (pH 7) with 0.1M $CaCl_2$ and 20% dextran (Sigma D 4133). The suspension is dropped through a hypodermic needle to form droplets which fall into rapidly stirred alginate solution. A capsular membrane forms almost instantaneously around the suspension drop due to the cross-linking of the interfacial alginate molecules by Ca^{+2} cations. Prior to the removal of the capsules the polymer solution is diluted five-fold by adding required amount of 0.1M HEPES buffer (pH 7). This step dilutes the alginate solution outside the capsules and reduces the possibility of capsules joining each other when they are in close contact, due to the gelation of the alginate solution on their exterior surface. Capsules are removed from the solution and excess solution is drained using an appropriate size mesh. The capsules are transferred to 0.1M HEPES buffer (pH 7) containing 0.1M $CaCl_2$ and incubated for one minute to stabilize the exterior surface. Finally capsules are equilibriated with the desired media.

EXAMPLE 2

This example illustrates formation of agarose capsules containing the desired material. A solution containing 0.5% agarose (Sigma A 4018, type VII) and 0.25% sodium alginate (Sigma A 7128, type IV) is prepared and kept warm and stirred using a magnetic stirrer at 40°C. An aqueous buffered suspension containing the biological material is prepared with 0.1M $CaCl_2$. The viscosity of this suspension is increased by adding 20% dextran (Sigma D 4100). The suspension is dropped through a hypodermic needle to form droplets which fall into the alginate/agarose solution. A capsular membrane forms almost instantaneously around the suspension drop due to the cross-linking of the interfacial alginate molecules by Ca^{+2} cations. The formed capsules are separated and shaken vigorously in oil medium at 35°C whereupon agarose in and around the membrane surrounding the

drop solidifies and the interfacial tension at the gel-oil interface gives rise to a smooth and uniform exterior surface. The capsules are then equilibriated in a buffer containing 0.05M EDTA, which is Ca^{+2} chelating agent, which liquifies and removes Ca-alginate component of the gel membrane. Finally capsules are washed and placed in the desired media.

The capsules thus formed were found to be reasonably strong due to the presence of agarose in the membrane matrix. The capsules were also found to be stable in solutions containing high concentrations of NaCl, EDTA, Phosphate etc.

EXAMPLE 3

This example illustrates the formation of chitosan capsules containing the desired material. 0.5% chitosan (Sigma C 3646) is dissolved in water containing 0.5% (v/v) acetic acid. Material to be encapsulated is mixed with 1.5% sodium-tri-poly- phosphate solution (pH 5.5) containing 40% dextran (Sigma D 4133). This suspension is extruded through a hypodermic needle connected to an air-jet for generating small droplets (0.5 - 1.0 mm diameter) of the viscous suspension. Droplets instantly form a chitosan polyphosphate membrane enclosing the droplet. Capsules are removed from the solution and further treated in 1.5% sodium-tri-poly- phosphate solution (pH 8.5) for a half hour. Finally the capsules are equilibriated in the desired buffer.

EXAMPLE 4

This example illustrates encapsulation of mammallian cells in alginate/poly-(L-lysine) capsules. KB cells are suspended in a solution consisting of 10% dextran, 1.3% $CaCl_2$ buffered with 13mM HEPES (pH 7) at a concentration of 10^5 cells/ml. The solution is extruded through an atomizer into rapidly stirred 0.25% sodium alginate solution (KELCO, LV) in isotonic NaCl solution. The capsules containing KB cells thus formed are removed after diluting the solution five fold using isotonic NaCl solution. The capsules are subsequently exposed to a 0.05% poly (L-lysine) solution for 5 minutes to strengthen the capsules. Finally capsules are removed and washed with isotonic solution to remove extra poly (L-lysine) before

equilibriating with the desired media. Cells encapsulated using this method remain viable and show normal growth.

Industrial Applicability

Encapsulation processes are finding increasing use in a variety of areas, particularly in biotechnology. Such process are used to encapsulate various materials such as enzymes, hormones, drugs, adsorbents and cells which can then be used in bioreactors, artificial organs, bioseparation systems, controlled drug-release systems, and so forth.

What Is Claimed Is:

1. A process for encapsulating material within a capsular membrane comprising the steps of:

(A) suspending said material in a medium which comprises an effective amount of gelling inducer;

(B) forming said suspension into a droplet containing said material, said droplet having an outer surface portion; and

(C) forming a discrete capsule by contacting said outer surface portion of said droplet with a gelling solution comprising an effective amount of a first gel forming polymer which gels upon contact with said gelling inducer to form a semi-permeable membrane.

2. The process of Claim 1 wherein said material is a biological material and said medium is an aqueous medium physiologically compatible therewith.

3. The process of Claim 2 wherein said first polymer is an ionotropic gel forming polymer and said membrane is subsequently coated with a polyelectrolyte polymer having a charge opposite to said first polymer to form a polyelectrolyte complex therewith.

4. The process of Claim 3 wherein said gelling inducer is a polyvalent ion.

5. The process of Claim 4 wherein said aqueous medium comprises a viscosity enhancer.

6. The process of Claim 5 wherein said biological material is selected from the group consisting of tissue, organelle, plant cells, animal cells, delta cells, whole islet of Langerhans,

hepatocytes, bacteria, algae, fungi, viruses, proteins and pharmaceutical compounds.

7. The process of Claim 6 wherein said first gel forming polymer is a polysaccharide gum.

8. The process of Claim 7 wherein said gum is sodium alginate.

9. The process of Claim 8 wherein said gum is chitosan.

10. The process of Claim 8 wherein said polyelectrolyte gel forming polymer is polylysine.

11. The process of Claim 10 wherein an interior portion of said first polymer is subsequently solubilized and removed from said capsule thereby leaving a capsule membrane made of said polyelectrolyte complex.

12. A process for encapsulating material within a capsular membrane comprising the steps of:

(A) suspending said material in a medium which comprises an effective amount of a gelling inducer;

(B) forming said suspension into a droplet containing said material, said droplet having an outer surface portion;

(C) forming a discrete capsule by contacting said outer surface portion of said droplet with a gelling solution comprising an effective amount of a first gel forming polymer and a second gel forming polymer, said first gel forming polymer gelling upon contact with said gelling inducer to form a semi-permeable membrane and entrapping said second gel forming polymer therein; and

(D) gelling said second gel forming polymer.

13. The process of Claim 12 wherein said material is a biological material and said medium is an aqueous medium physiologically compatible therewith.

14. The process of Claim 13 wherein said first gel forming polymer is an ionotropic gel forming polymer and said gelling inducer is a polyvalent ion.

15. The process of Claim 14 wherein said second gel forming polymer is a thermal gel forming polymer.

16. The process of Claim 15 wherein said aqueous medium comprises a viscosity enhancer.

17. The process of Claim 16 wherein said biological material is selected form the group consisting of tissue, organelle, plant cells, animal cells, delta cells, whole islet of Langerhans, hepatocytes, bacteria, algae, fungi, viruses, proteins, and pharmaceutical compounds.

18. The process of Claim 17 wherein said ionotropic gel forming polymer is a polysaccharide gum.

19. The process of Claim 18 wherein said gum is sodium alginate.

20. The process of Claim 18 wherein said gum is chitosan.

21. The process of Claim 18 wherein said thermal gel forming polymer is agarose.

22. The process of Claim 18 wherein an interior portion of said first gel forming polymer is subsequently solubilized and removed from said capsule.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/02413

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 12 N 11/04; A 61 K 9/50; B 01 J 13/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 12 N; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Chemical Abstracts, vol. 79, no. 25, 24 December 1973 (Columbus, Ohio, US) see page 218, abstract 145072r, & JP, A, 7316183 (SNOW BRAND MILK PRODUCTS CO. LTD) 19 May 1973	1,2
Y	--	3-11
Y	Biotechnology and Bioengineering, vol. 27, no. 2, February 1985 John Wiley & Sons, Inc. (New York, US) M.F.A. Goosen et al.: "Optimization of microencapsulation parameters: semipermeable microcapsules as a bioartificial pancreas", pages 146-150, see pages 146-147, "Materials and methods. Microencapsulation of living cells"; page 147, "Dependence of microcapsule morphology on sodium alginate viscosity and purity" --	3-11

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* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10th October 1988	04 NOV 1988
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	US, A, 4409331 (FRANKLIN LIM) 11 October 1983 see the whole document	1-22

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8802413 SA 23613

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 25/10/88
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		FR-A,B 2503183	08-10-82
		SE-A- 8201554	14-09-82
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